REMARKS

Applicant wishes to thank Examiners Khatol S. Shahnan Shah and Mark Navarro for the courtesy extended to inventor Dr. Xin-Xing Gu and their representatives, Nancy Vensko, attorney of record, and Susan Ano, National Institutes of Health (NIH), Office of Technology Transfer (OTT), Technology Licensing Specialist, on April 29, 2003. The Interview Summary Form PTOL-413 summarizes the discussions held at the personal interview. The present response to the outstanding Office Action includes the substance of the Examiner Interview.

A. Disposition of Claims

Claims 39-50 are pending in the application. Claims 48-50 have been added. Support for the amendment is found throughout the specification, for example, for the addition of Claims 48 and 49 at 9:19-23 ("Although the use of hydrazine for detoxification of LOS from M catarrhalis is described herein, the use of any reagent or enzyme capable of removing esterified fatty acids from lipid A, such as mild alkaline treatment, i.e., treatment with dilute (0.1 N) NaOH or other dilute aqueous base solutions having a pH of between about 13.2 and 13.6, is within the scope of the present invention."), and for the addition of Claim 50 at 14:7-12 ("The toxicity of isolated LOS [non-detoxified], and dLOS [treated with hydrazine] were tested using the standard Limulus amebocyte lysate (LAL) assay ... The isolated LOS showed 20,000 EU/μg, whereas the dLOS showed 1 EU/μg, representing a 20,000-fold reduction of toxicity."). Additionally, per http://www.uspto.gov/web/offices/pac/dapp/opla/preognotice/benefitclaims.pdf, the Specification has been amended to include a proper claim to priority. Reexamination and reconsideration of the application, as amended, are respectfully requested.

B. Scope of Claims

The claims are related to the discovery that hydrolysis of esterified fatty acids detoxifies lipooligosaccharide (LOS) from *Moraxella catarrhalis* without destroying the immunostimulatory activity. Two approaches for detoxification of lipopolysaccharide (LPS) or LOS have been applied to obtain clinically acceptable polysaccharide (PS) or oligosaccharide (OS) from LPS or LOS.[1] Mild-acid treatment of LOS cleaves the lipid A portion from the LOS

[1] Gu et al., I&I 66:1891, May 1998, of record, at p. 1895, paragraph bridging col. 1 and col. 2.

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molecule.[2] Mild alkali treatment (or enzymatic treatment) of LOS removes ester-linked fatty acids while preserving attachment of lipid A.[3] By way of illustration, Munford et al. (U.S. Pat. No. 5,013,661 issued May 7, 1991), of record, at Fig. 2, has been adapted to show LPS from *Salmonella typhimurium*, *Escherichia coli*, *Haemophilus influenza*, and *Neisseria meningitidis* that has been detoxified in one approach by mild alkali treatment to remove primary ester-linked fatty acids (*) and secondary ester-linked fatty acids (↓) and that has been detoxified in another approach by enzyme treatment to remove secondary ester-linked fatty acids (↓) only:

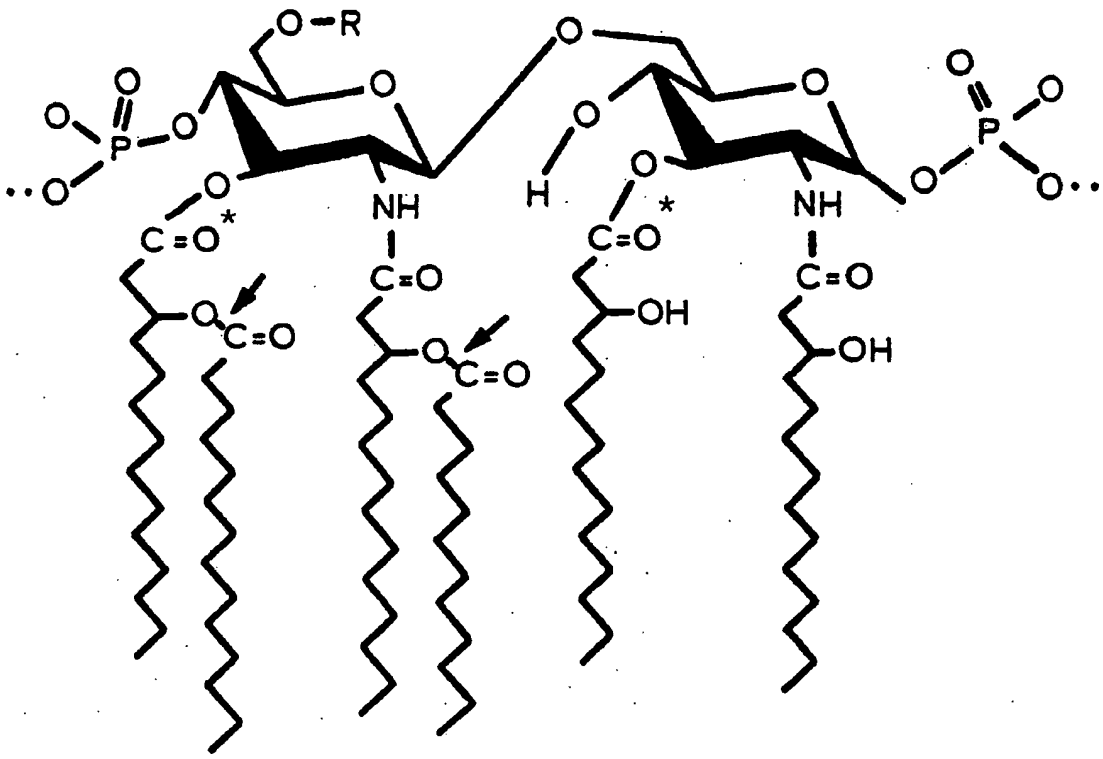

Fig. 2

[2] *Id.*
[3] *Id.*

Applicant used mild-alkali treatment as opposed to mild acid treatment for the detoxification of *M. catarrhalis* because the resulting dLOS surprisingly gave a better yield and immunogenicity after conjugation to proteins carriers (Examples 9-11).[4] As stated in the Specification at 9:19-23: "Although the use of hydrazine for detoxification of LOS from *M catarrhalis* is described herein, the use of any reagent or enzyme capable of removing esterified fatty acids from lipid A, such as mild alkaline treatment, i.e., treatment with dilute (0.1 N) NaOH or other dilute aqueous base solutions having a pH of between about 13.2 and 13.6, is within the scope of the present invention." The discovery that hydrolysis of esterified fatty acids detoxifies LOS from *M. catarrhalis* without destroying the immunostimulatory activity extends to detoxification by mild alkali treatment, e.g., by hydrazine, and enzymatic treatment, because detoxification of LOS by mild alkali treatment, e.g., by hydrazine, and enzymatic treatment all produce hydrolysis of esterified fatty acids while preserving attachment of lipid A as opposed to mild-acid treatment of LOS that cleaves the lipid A portion from the LOS molecule.

In a preferred embodiment, as stated in the Specification at 14:7-12: "The toxicity of isolated LOS [non-detoxified], and dLOS [treated with hydrazine] were tested using the standard Limulus amebocyte lysate (LAL) assay ... The isolated LOS showed 20,000 EU/μg, whereas the dLOS showed 1 EU/μg, representing a 20,000-fold reduction of toxicity." According to W.H.O. Expert Committee on Biological Standardization, WHO Tech. Rep. Ser. 814:15, 1991, of record, at page 22, an endotoxin content, at least for *H. influenzae*, shall be less than 10 International Units of Endotoxin per μg when measured by a Limulus amebocyte lysate (LAL) test. Detoxification of *M. catarrhalis* LOS with hydrazine in the preferred embodiment resulted in a 20,000-fold reduction in the level of endotoxin so that it showed 1 EU/μg, making it quite clinically acceptable.[5]

Antibodies elicited by LOS-based conjugate vaccines were bactericidal (Example 6, published as Gu et al., I&I 66:1891, May 1998, of record) and enhanced clearance of *M. catarrhalis* from the lungs of mice (Example 7, published as Hu et al., I&I 68:4980, Sept 2000, of record). A monoclonal antibody against *M. catarrhalis* LOS showed that epitopes of LOS are surface expressed (Hu et al., I&I 69:1358, Mar 2001, attached). Figure 3 of Hu et al., 2001,

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[4] *Id.*
[5] *Id.*

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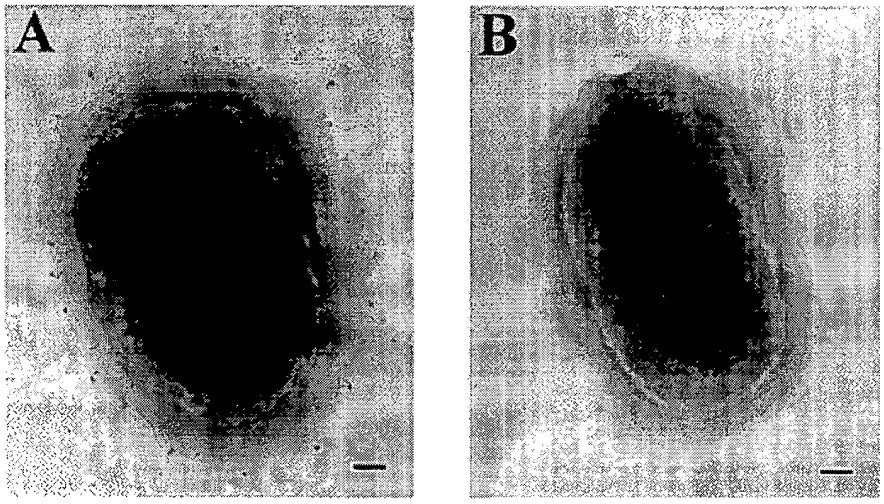
shows binding of the monoclonal antibody to the surface of *M. catarrhalis* as visualized by immuno-electron microscopy, where A is incubation with the monoclonal antibody, and B is incubation with irrelevant ascites:



Nasal immunization by LOS-based conjugate vaccines resulted in an effective immune response and protection against challenge in a mouse pulmonary clearance model (Jiao et al., I&I 70:5982, Nov 2002, attached), extending the previous findings of Gu et al. to this important alternative route of administration. In sum, the scope of enablement is commensurate with that of the claims.

C. Compliance with 35 U.S.C. 103(a)

The Office Action rejected the claims under 35 USC 103(a) as being unpatentable over Gu et al., U.S. Pat. No. 6,207,157 filed Apr 23, 1997, in view of Vaneechoutte et al., Journal of Clinical Microbiology 28:182, 1990, Campagnari et al., Microbial Pathogenesis 8:353, 1990, and Edebrink et al., Carbohydrate Research 257:269, 1994. Gu et al. describes a conjugate vaccine for nontypeable *Haemophilus influenzae* comprising lipooligosaccharide (LOS) from which esterified fatty acids have been removed to form detoxified LOS (dLOS) conjugated to an immunogenic carrier. Vaneechoutte et al. describes antibody-reactive compositions comprising

lipooligosaccharides isolated from *Moraxella (Branhamella) catarrhalis*. Campagnari et al. shows that lipooligosaccharide epitopes are shared among Gram-negative non-enteric mucosal pathogens, examples of which include *M. catarrhalis*, *H. influenzae*, and *Neisseria meningitidis*. Edebrink et al. describes structural studies of the O-polysaccharide from the lipooligosaccharide of *M. catarrhalis* and characterizes the LPS of *M. catarrhalis* as lacking the extended O-antigenic side chains characteristic of enteric pathogens, thus being similar in general structure to the LPS of *N. meningitidis*, *Neisseria gonorrhoeae*, *H. influenzae*, and *Bordetella pertussis*. It is the Office's contention that the combination of these references would make the development of the instant invention obvious to one skilled in the art. According to the MPEP 2143: "To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations." Additionally, MPEP 716.02(a) states that "Greater than expected results are evidence of non-obviousness." In light of these criteria, Applicant makes three arguments for the non-obviousness of the instant invention.

First, the invention shows unexpectedly superior results than those reported in the art. (To establish a case of obviousness, a reasonable expectation of success must be found in the prior art, *not* in applicant's disclosure. MPEP 2143. Here, the unexpectedly superior results are found in applicant's disclosure.) Neither Vaneechoutte et al. (phenol extraction, at abstract) nor Campagnari et al. (phenol extraction, at p. 361) provided evidence that LOS obtained following their methods (phenol extraction) would induce bactericidal activity of immune antisera against *M. catarrhalis*. As for Edebrink et al., the polysaccharide of *M. catarrhalis* LPS was prepared by mild acid treatment (at abstract). In contrast, the patent specification shows in Figure 1 (reproduced below) that dLOS conjugates detoxified by mild-alkali hydrolysis would indeed induce bactericidal activity of immune antisera against *M. catarrhalis*. Figure 1 also shows that dLOS conjugates are dramatically more effective than LOS (as would be obtained following the phenol extraction methods of Vaneechoutte et al. and Campagnari et al.) in inducing bactericidal activity of immune antisera against *M. catarrhalis*. Mild-alkali hydrolysis for the detoxification

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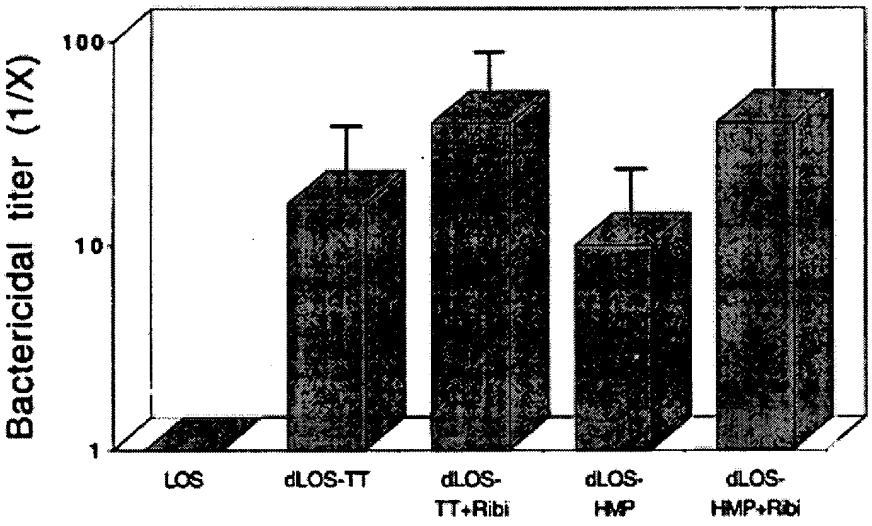
of *M. catarrhalis* LOS was surprisingly superior to the mild-acid treatment of Edebrink et al because the resulting dLOS gave a better yield and immunogenicity after conjugation to protein carriers (Examples 9-11). These side-by-side comparisons confirm greater than expected results:



Second, the immunogenicity of detoxified LOS is unpredictable, which is repeatedly taught in the art. Gupta et al., I&I 60: 3201, 1992, of record, shows that LPS isolated from *V. cholerae*, detoxified by alkaline treatment with hydrazine, and conjugated to a carrier protein results in a conjugate that raises bactericidal antibodies, i.e., is potentially a vaccine. Polotsky et al., I&I 62: 210, 1994, of record, describes LPS isolated from *S. flexneri* and detoxified by treatment with either acetic acid producing O-SP (to cleave lipid A from the OS) or with hydrazine producing deALPs (to remove primary O-linked fatty acids), and shows that coupling to a carrier protein results in both conjugates being immunogenic and raising bactericidal antibodies. However, the O-SP conjugates were more immunogenic, and the hydrazine-detoxified LPS from Shigella was poorly immunogenic. Moreover, these experts state at the last sentence, "These experimental data indicate that it is not yet possible to predict the immunogenicity of the saccharide component of newly devised conjugates by measurement of its molecular weight or by its ratio to protein." This last sentence means that a leading group in the field cannot tell whether acid or hydrazine treatment will result in a detoxified LOS or LPS that retains its immunogenicity. Konadu et al., I&I 62: 5048,

1994, of record, shows *E. coli* O157 LPS being detoxified by acid treatment or by hydrazine treatment, both conjugates raising similar levels of bactericidal antibodies, with the acid treated conjugates being slightly although not statistically better. Gupta et al., I&I 63: 2805, 1995, of record, compares different routes of detoxification of *E. coli* O111 LPS, in this case hydrazine detoxification and the use of an adipic dihydrazide linker giving the best results. Again, the same experts, at the bottom of the left column of page 2809, state: "It is not yet possible to predict the immunogenicity of the saccharide component by in vitro methods, so it will be necessary to compare the immunogenicity analyses of new conjugates." In other words, retaining immunogenicity while reducing toxicity is experimental. Finally, Konadu et al., I&I 64: 2709, 1996, of record, compares acid and hydrazine detoxification of LPS from *S. paratyphi* A, and shows that hydrazine treatment results in conjugates that do not raise bactericidal antibodies, while acid treatment does. In sum, this body of work shows that the contribution of detoxification to immunogenicity cannot be predicted in advance. Consequently, this showing negates any reasonable expectation of success in Applicant's treatment of LOS. *In re O'Farrell*, 7 USPQ2d 1673, 1681 (Fed. Cir. 1988) (Obviousness under § 103 requires "a reasonable expectation of success."). Applicants' success in detoxifying LOS from *M. catarrhalis* while retaining its immunogenicity was empirical and nonobvious.

Third, there is no suggestion in the art to select Applicant's treatment of LOS. The Office cited Campagnari et al. as showing that LOS epitopes are shared among Gram-negative non-enteric mucosal pathogens, which include *H. influenzae* and *M. catarrhalis*. However, Campagnari et al. in no way teaches that the epitopes remaining after detoxification of LOS would be shared. To the contrary, past attempts to remove esterified fatty acids had shown that the contribution to immunogenicity of such deacylation could not be predicted in advance. Erwin et al., I&I 59: 1881, 1991, of record, studied the impact of enzyme deacylation on the bioactivities of several LPS materials. The enzymatically deacylated LPS from *E. coli, H. influenzae, N. meningitidis,* and *S. typhimurium* were similarly reduced in potency in the *Limulus* toxicity test, 30- to 60-fold reduction in potency relative to the corresponding mock-treated LPS (p. 1883, col. 1, ¶ 1). However, while the mitogenicity of enzymatically deacylated LPS from *E. coli, H. influenzae,* and *S. typhimurium* was reduced 15-fold, the mitogenicity of *Neisseria* LPS was reduced 100-fold by enzyme deacylation (p. 1883, col. 1, ¶ 2). This incongruity caused Erwin et al. to hypothesize

that deacylation could lead to different structural alterations for one LPS compared to another LPS (p. 1885, col. 1, ¶ 2). Erwin et al. reasoned that deacylation might result in a conformational change in the LPS. *Id.* Because the binding of antibodies is conformationally dependent, this possibility caused Erwin et al. to posit that deacylation might block immunogenicity. *Id.* The conclusion from Erwin et al. is that the contribution of deacylation to the bioactivity of a given LPS cannot be predicted with confidence from the reported structure-activity relationships of lipid A or from the behavior of other deacylated LPS (abstract, last line; p. 1884, col. 2, ¶ 1; p. 1886, col. 1, ¶ 2). Accordingly, there is no reason or suggestion in the art to select Applicant's treatment of LOS, deacylation, when the art of Erwin et al. indicates that it was uncertain whether it could have been used successfully. *In re Dow Chemical Co.*, 5 U.S.P.Q.2d 1529, 1532 (Fed Cir. 1988) ("There must be a reason or suggestion in the prior art for selecting the procedure used.").

Based on these three points, it is clear that the instant invention is not obvious and rejection of the pending claims should be withdrawn.

CONCLUSION

In view of the above, it is submitted that the claims are in condition for allowance. Reconsideration and withdrawal of all outstanding rejections are respectfully requested. Allowance of the claims at an early date is solicited. If any points remain that can be resolved by telephone, the Examiner is invited to contact the undersigned at the below-given telephone number.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 6/2/03

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